Escape from fatal fusion

In 1971, a seminal paper from the laboratory of Philip D'Arcy Hart reported that the intracellular pathogen *Mycobacterium tuberculosis* avoids destruction in the cell's lysosomes by avoiding these organelles altogether—a trick now known to be used by many other intracellular pathogens.

At the age of 65, when most people are thinking of retiring, Hart was beginning a new phase of his career. As head of the MRC Tuberculosis Research Unit, London, UK, Hart had already become famous for designing what is still the definitive format for a clinical trial. Now, Hart wanted to understand the biology of tuberculosis infection. Retirement afforded him the time he needed to spend at the bench.

Dodging digestion

It was known that microbes entering the body were generally gobbled-up and disposed of by roaming macrophages. But it was also clear that certain microbial pathogens, including *M. tuberculosis,* were able to survive and multiply inside these cells, leading to prolonged, chronic infection. When Hart started his laboratory work at the National Institute of Medical Research in London, UK, nothing was known about how these pathogens survived their intracellular fate.

Phagosomes containing inert particles had been shown to transit from the cell surface and fuse with "dense granules" (lysosomes) (1), and biochemical studies had revealed that bacteria could be degraded by lysosomal enzymes (2). This led Hart to hypothesize two possible survival tactics for *M. tuberculosis*: either the bacteria was resistant to lysosomal enzymes, or it somehow avoided contact with them.

Hart combined electron microscopy with the use of an electron-opaque tracer to mark the lysosomes. This novel approach enabled him and coworker Johnathan Armstrong to report in the *Journal of Experimental Medicine*, in 1971, that *M. tuberculosis* avoids death-by-digestion by remaining in a phagosome compartment that is separate from the lysosomes (3).

What works for one...

The paper set off a chain of similar reports: a year later two groups reported similar observations for *Chlamydia* bacteria (4) and the protozoan *Toxoplasma gondii* (5). And by the following decade *Legionella pneumophila* and *Encephalitozoon cuniculi* had joined the list of pathogens that escape a lysosomal fate (6, 7).

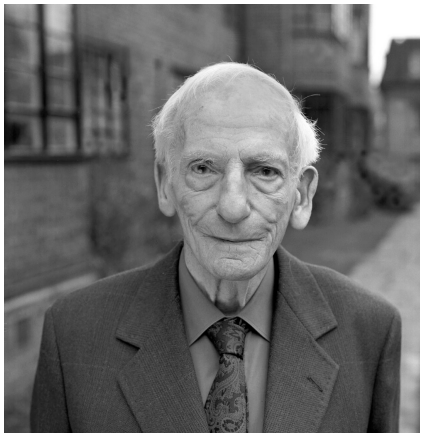
The paper also marked a turning point for cellular microbiology. "Intracellular parasites now weren't just inside cells," says Marcus Horwitz, University of California, Los Angeles. "You could now begin to say how their pathways differed." Reports by Horwitz published in the *Journal of Experimental Medicine* showed that, whereas *L. pneumophila* establishes its own unique phagosome sanctuary, displaying characteristics of the rough endoplasmic reticulum (8), *M. tuberculosis* inhibits phagosome maturation. Phagosomes normally adopt membrane markers of early and then late endosomes before finally fusing with lysosomes. *M. tuberculosis*–containing phagosomes, however, halt at the early endosome stage (9).

Location matters

Hart's discovery has also been shaping the path of drug and vaccine design. David Russell, Cornell University College of Veterinary Medicine, New York, NY, is interested in designing drugs against intracellular pathogens and says that, to do so effectively, "you have to appreciate the environment in which the bacterium finds itself."

As for vaccines, Horwitz explains that the intracellular location of a microbe determines the manner in which its proteins are displayed to the immune system. So when designing a new vaccine for *M. tuberculosis*, Horwitz ensured its delivery into phagosomes in order to mimic the real bug.

This new vaccine is now being tested in human clinical trials—trials which will adhere to the rigorous design established by Hart in his prelaboratory days.



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Philip Montagu D'Arcy Hart

Philip Montagu D'Arcy Hart (1900–2006)

By sad coincidence Philip D'Arcy Hart died on Sunday, July 30, while this article was being prepared. Hart's work on cellular microbiology represents a small fraction of his extensive contribution to medical research (10) in a career that began in the 1920s and continued, with regular visits to the lab, until a few years ago.

David Russell, a friend and colleague, described Hart as having unrelenting energy for science. "He clearly loved what he did, and his enthusiasm was deeply infectious," he said. "I still smile every time I reference his papers."

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